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REVIEWS

Cannabinoids as potential new therapy for the treatment of gliomas

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Gliomas constitute the most frequent and malignant primary brain tumors. Current standard therapeutic strategies (surgery, radiotherapy and chemotherapeutics, e.g., temozolomide, carmustin or carboplatin) for their treatment are only palliative and survival diagnosis is normally 6–12 months. The development of new therapeutic strategies for the management of gliomas is therefore essential. Interestingly, cannabinoids have been shown to exert antiproliferative effects on a wide spectrum of cells in culture. Of interest, cannabinoids have displayed a great potency in reducing glioma tumor growth either *in vitro* or in animal experimental models, curbing the growth of xenografts generated by subcutaneous or intratecal injection of glioma cells in immune-deficient mice. Moreover, cannabinoids appear to be selective antitumoral agents as they kill glioma cells without affecting the viability of nontransformed counterparts. A pilot clinical trial on patients with glioblastoma multiforme demonstrated their good safety profile together and remarkable antitumor effects, and may set the basis for further studies aimed at better evaluating the potential anticancer activity of cannabinoids.

KEYWORDS: angiogenesis • apoptosis • cannabinoid • clinical trial • endocannabinoid system • glioma • tumor growth

The endocannabinoid system

Preparations from *Cannabis sativa* (marijuana) have been used for many centuries both medically and recreationally. The plant contains over 400 chemical entities including alkaloid derivatives of spermidine, sterols, terpenes and flavonoid glucosides, and approximately 70 cannabinoids that are predominantly found in the flowering tops of the plant, possessing either psychoactive or non psychoactive properties. However, the chemical structure of their active components, the cannabinoids, was not elucidated until 1964 when Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the most relevant psychoactive molecule, was isolated in a pure form. The compounds are highly hydrophobic and they were initially thought to act by interacting with biomembranes [1].

The long standing issue of the mechanism of action of cannabinoids was solved with the important discovery of cannabinoid receptors in the early 1990s. Soon after, the existence of endogenous cannabinoid receptor ligands (endocannabinoids), the most important of

these being anandamide (arachidonoyl ethanolamide, AEA) and 2-arachidonoyl glycerol (2-AG) was demonstrated; it was also found that the greater part of cells possess specific degrading enzymes and membrane transport proteins for these compounds. The arachidonic acid derivatives (AEA and 2-AG) are exciting discoveries, not just for depicting a new “endocannabinoid system”, but because they also represent a novel class of modulators derived from membrane fatty acids that may be very important in neuromodulation, as well as in brain-immune axis regulation, mimicking in a similar way the pan-action already described for opioid endogenous system. This evidence suggests a novel modulatory and ubiquitous system whose the physiological role forewarns to be complex and widespread [2–4].

To date, two main subtypes of cannabinoid receptors have been identified, designated cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2) [2,3]. Although CB1 is predominantly expressed in the brain, it has also been detected in the periphery. Conversely, the CB2 receptor subtype appears to be the principal

form of cannabinoid receptor within the immune system, with a low quantifiable expression in healthy brain. They are coupled to Gi/Go proteins through which they inhibit the adenylyl cyclase. The CB1 receptors also modulate ion channels, inducing inhibition of voltage-sensitive Ca^{2+} channels and activation of G-protein-activated inwardly rectifying K^+ channels [2,3]. Cannabinoid receptors also modulate several signaling pathways that are more directly involved in the control of cell proliferation and survival, including ERKs [5], c-Jun N-terminal kinase, p38 MAPK [6,7], PI3K/Akt [8], focal adhesion kinase [9] and synthesis of ceramide [10].

Besides the well-known psychotropic effects of cannabis and its use as an illicit drug, recent studies have examined the potential application of cannabinoids as therapeutic agents. One of the most active and promising areas of current research in the cannabinoid field is the study of the potential application of cannabinoids as antineoplastic drugs.

The beneficial effects of cannabinoids on some cancer-related disorders, such as emesis, nausea, depression, chronic pain and appetite suppression, have been known to exert palliative effects in cancer patients since the early 1970s [11], the best established of these effects being the inhibition of chemotherapy-induced nausea and vomiting. Although cannabinoids showed some antiemetic efficacy, they are unlikely to be used as first-line treatment for vomiting and nausea associated with chemotherapy; however, newer and more effective agents may have a role as co-antiemetic treatment using . To date, Δ^9 -THC (Dronabinol[®] and marinol[®]) and its synthetic derivative nabilone (Cesamet[®]) can be legally prescribed in the USA for the treatment of nausea and vomiting for cancer patients undergoing chemotherapy and for the stimulation of appetite for patients with AIDs.

However, in the last few years, the most exciting result from emerging major studies published between 2000–2006 is the ability of cannabinoids to fight cancer cells. We now know that cannabinoids arrest and/or reduce many types of cancer growth (e.g., brain, breast, leukemic, melanoma, pheochromocytoma, pancreatic and thyroid) through promotion of programmed cell death (apoptosis), which is lost in tumors, and by arresting angiogenesis and cancer cell migration [12–15]

Cannabinoids in gliomas

Malignant gliomas remain the most deadly human brain tumors, with poor prognosis despite years of research in antitumoral therapeutic strategies. The historical median survival of patients with a glioblastoma multiforme (GBM) using the best radiological, surgical and anticancer drug therapy available is less than 1 year [16]. A hallmark characteristic of gliomas is their molecular and cellular heterogeneity (either in terms of pathology and genetic changes even within a single tumor) which is thought to be one of the reasons for their malignancy and recurrency [16,17]. A large number of chemotherapeutic agents (e.g., alkylating agents such as temozolomide and nitrosureas such as carmustine) have also been tested, but no remarkable

improvement in patient survival has been achieved as yet. The use of therapeutic adjuvants to surgical resection, such as focal radiotherapy and chemotherapy, provides only a negligible improvement in the disease course and life expectancy. The toxicity profile of these protocols is variable with myelosuppression being the most frequent and limiting factor. At present, adjunctive chemotherapy (concurrent temozolomide with radiation followed by 6 months of monthly temozolomide) improves median survival by 2.5 months compared with radiation therapy alone [18]. Thus, temozolomide has become a standard adjuvant therapy for malignant gliomas. Despite this multimodality treatment, clinical recurrence or progression is nearly universal. Intracavitary carmustine wafer implantation in surgically respectable cases of recurrent GBM provided only an 8-week survival benefit [19]. Available systemic chemotherapies offer modest clinical benefit with a 6-month progression-free survival of less than 15% for GBM and 31% for anaplastic astrocytoma. Likewise, although immunotherapy strategies appear promising as a new and safe approach to induce an antitumor immune response [20], no immunotherapy or gene therapy trial performed to date has been significantly successful. Taken together, novel therapies for these devastating tumors remain an unmet need.

For these reasons, in 1998, the first Guzman's group report describing the antitumoral effect of Δ^9 -THC in C6 murine glioma cells was welcomed as an important paper [21]. It was shown that THC-induced glioma cell death was not mediated by the CB1 cannabinoid receptor stimulation and accompanied by a significant breakdown of cellular sphingomyelin. Later, the same research group demonstrated that, following CB1 receptors activation, two peaks of ceramide generation are observed with different kinetics (minute vs day range), magnitude (two- vs fourfold), mechanistic origin (sphingomyelin hydrolysis vs ceramide synthesis *de novo*) and function (metabolic regulation vs induction of apoptosis) [22–24]. Moreover, Carracedo *et al.* [25] by using a wide array of experimental approaches, identified the stress-regulated protein p8 as an essential mediator of cannabinoid antitumoral action and showed that p8 up-regulation is dependent on the *de novo*-synthesized ceramide [25]. p8 upregulation also occurs *in vivo* and resistance to cannabinoid treatment is associated with decreased activation of the p8-regulated proapoptotic pathway [25].

In vivo studies using animal models showed that local administration of THC or the synthetic cannabinoid WIN-55,212–2 reduced the size of tumor generated by intracranial inoculation of C6-derived glioma in Wistar rats [22] with a concomitant involvement of CB1 and CB2 receptors. Moreover, rats with malignant gliomas, when treated intratumorally with cannabinoids, survived significantly longer than untreated animals and showed a complete eradication of the tumors, as evidenced in 20–35% of treated animals. In order to rule out the possible implication of the immune system in this action, similar experiments on mice deficient in *RAG2*, which lack mature T and B cells were performed. The growth of the glioma was significantly lower in cannabinoid-treated mice, although, in this case,

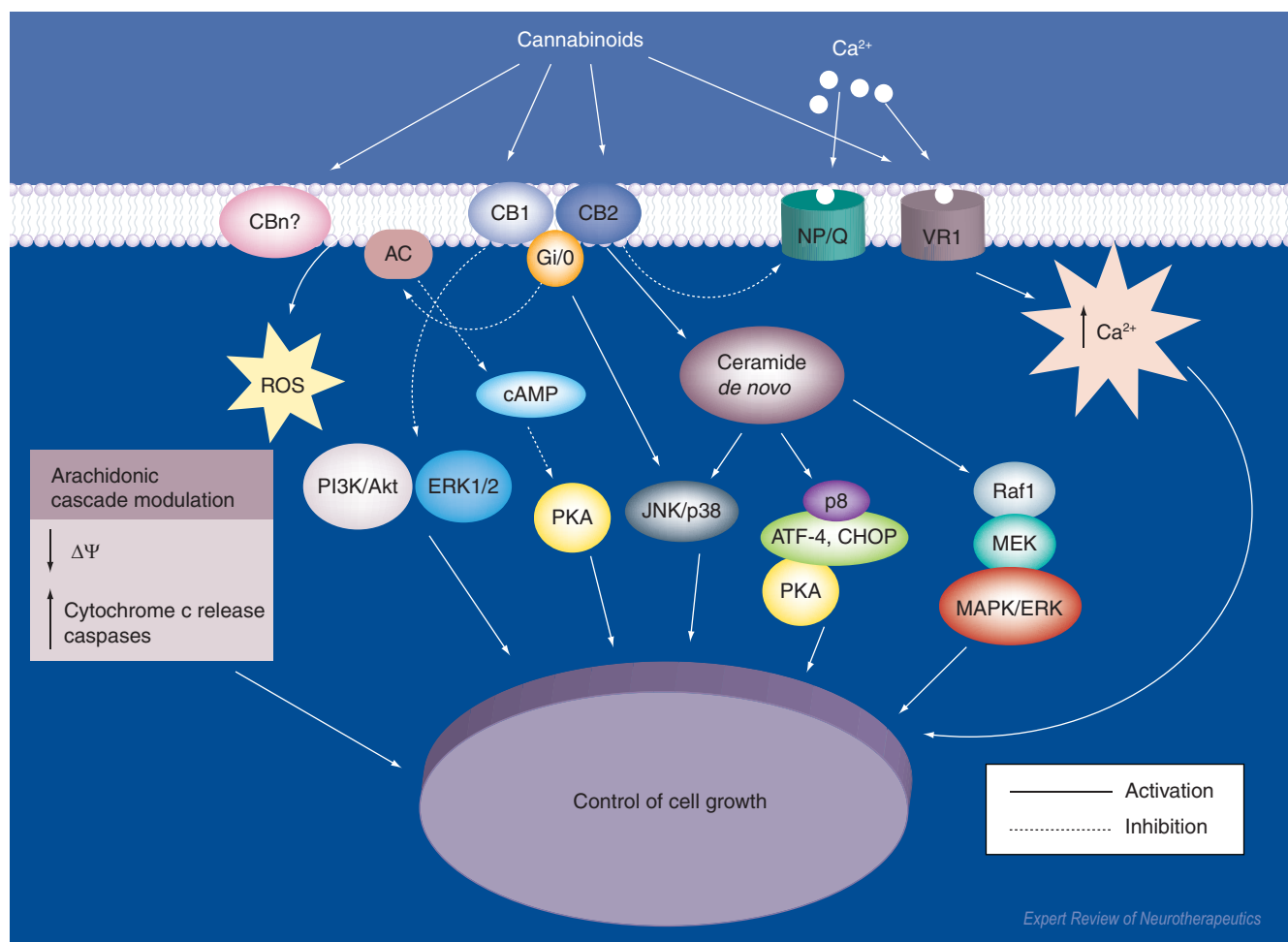


Figure 1. Signaling pathway activated by cannabinoids in the control of cancer cell growth. Natural, synthetic and endogenous cannabinoids exert their anti tumoral effects through various cellular pathways by binding to specific CB1 and CB2 Gi/o-protein coupled receptors (or unknown CBn receptor), as well as VR1. CB receptor signal triggers the inhibition of the activity of adenylate cyclase (AC). The decrease in cAMP levels inhibits the protein kinase (PKA) pathway determining an inhibition of tumor cell cycle. CB receptors can also indirectly inhibit voltage-sensitive Ca²⁺ channels (N and P/Q type), as well as promote Ca²⁺ release from intracellular stores, thus controlling cell fate. The stimulation of CB receptors may lead to an increase in ceramide levels mainly through synthesis *de novo*. Ceramide accumulation can cause upregulation of p8 and in turn upregulation of the ER stress-related genes *ATF-4*, *CHOP* and *TRB3*. Ceramide also activates Raf1/mitogen-activated protein kinase (MEK)/ERK cascade and JNK. The activation of these pathways results in inhibition of cell proliferation. By contrast, since the role of ERK in controlling cell fate is very complex, cannabinoids can also inhibit ERK, as well as the PI3k/AKT pathway, thus promoting apoptosis. Alternatively, cannabinoids can trigger activation of ROS, elevation of intracellular Ca²⁺, modulation of the arachidonate cascade, inhibition of mitochondrial potential (ΔΨ), increase in cytochrome c release and activation of apoptotic caspases. The crosstalk between the different pathways and many molecular details have been omitted for simplification.

AC: Adenylate cyclase; CB: Cannabinoid; CBn: Unknown cannabinoid receptor; ER: Endoplasmic reticulum; ERK: Extracellular signal-regulated kinase; ROS: Reactive oxygen species; VR: Vanilloid receptor

total eradication was not observed [22]. These findings suggest that cannabinoids exert their antiproliferative action with a specific mechanism on tumor cells, although the effects may be reinforced by an immune reaction. However, the role of the immune system in the antitumor effects of cannabinoids in gliomas is far from clear and requires further investigations.

Remarkably, the antiproliferative effect of cannabinoids appears to be selective for tumor cells, as the survival of normal brain cells is unaffected or even favored by cannabinoid challenge

[26,27], supporting the notion that cannabinoid receptors regulate cell survival and cell death pathways differently in tumor and nontumor cells (see later).

CB2 agonists & gliomas

Although cannabinoids have a favorable drug safety profile, their use in clinic can be severely impaired by their psychoactivity and psychotropic side effects, raising a number of clinical

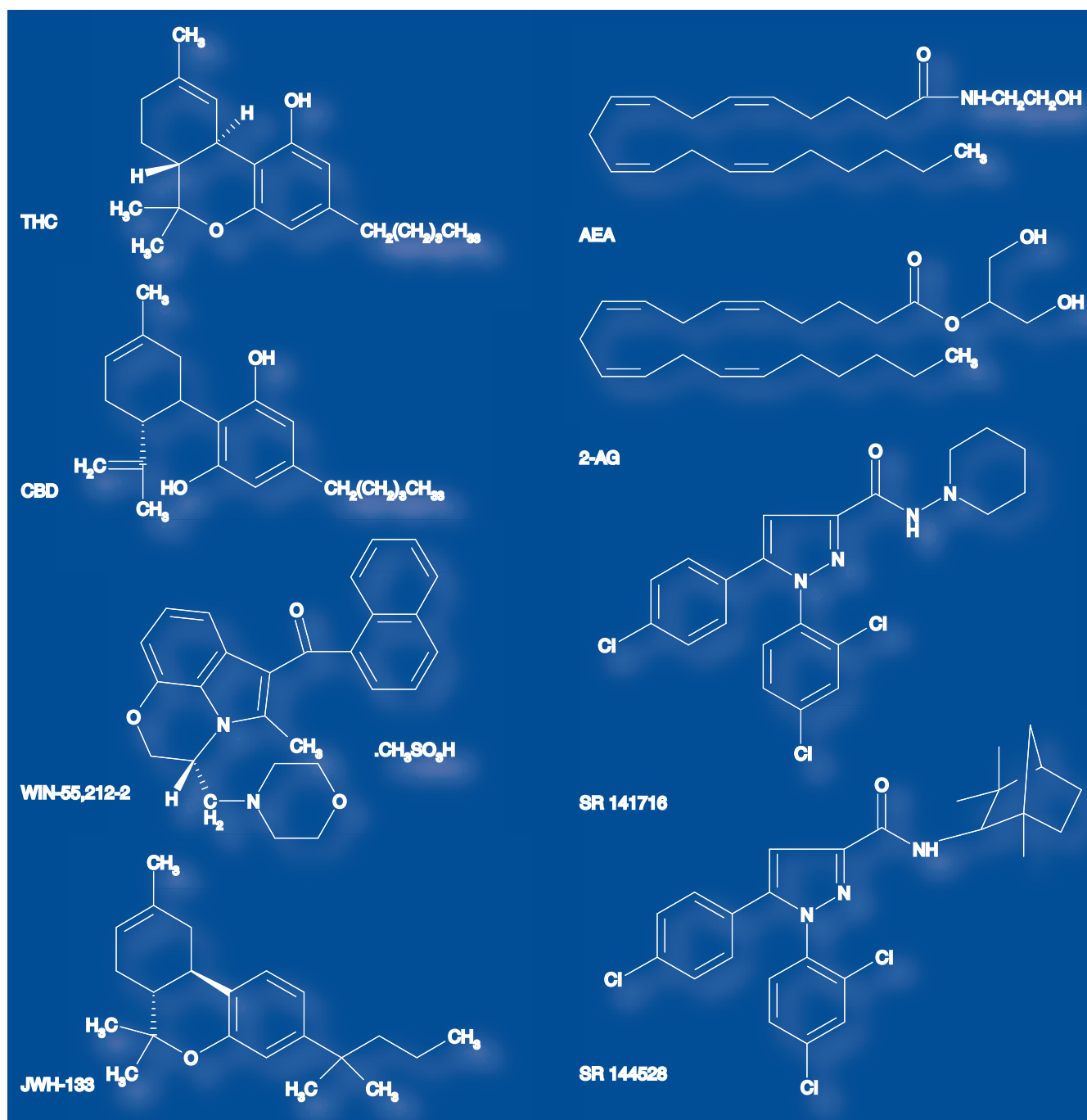


Figure 2. Chemical structure of cannabinoids. Chemical structure of the psychotropic plant-derived Δ9-tetrahydrocannabinol (Δ9-THC), the nonpsychotropic cannabidiol (CBD), synthetic WIN-55,212-2, synthetic selective CB2 compound JWH-133 and the principal endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The selective CB1 receptor antagonist SR141716A and the CB2 receptor antagonist SR 144528 are also represented. CB: Cannabinoid.

and ethical considerations. These adverse effects are within the range of those accepted for other medications, especially in cancer treatment, and tend to disappear with tolerance following continuous use, but cannabinoid-based therapies devoid of side effects would be desirable.

The unwanted psychotropic effects of marijuana-derived cannabinoid are mediated largely or completely by neuronal CB1 receptors. Thus, great efforts have been made to assess alternative possibilities. One of the most obvious strategies to avoid psychotropic side effects in the management of glioma tumor growth is

the administration of CB2-selective compounds. The CB2 cannabinoid receptor subtype is expressed preferentially in peripheral tissues, particular in the immune system, with low expression in the healthy brain. For this reason, selective agonists for this receptor subtype are devoid of psychoactivity, although it should be kept in mind that the CB2 receptor mediates the immunosuppressant effect of cannabinoids that would play against the organism's own defense against the tumor [3].

The recent evidence that CB2 receptors are present in both cultured neurons and the nervous system has to be taken in account [2,3]. For example, healthy neurons and glial cells under inflammatory conditions show detectable levels of the receptor [28–31]. Moreover, the coexpression of the CB1 and CB2 cannabinoid receptors has been detected in rat C6 glioma cells [32] and in biopsies from human astrocytomas [23], using both western blot and immunohistological methods. Almost 70% of human astrocytomas express significant levels of both cannabinoid receptors and the extent of CB2 receptor expression appears to be related to tumor malignancy. In this context, it has been demonstrated that the local and *in vivo* daily administration of the selective CB2 agonist JWH-133 in mice bearing subcutaneous glioma caused a considerable regression of malignant tumors, inducing a classical pattern of apoptosis via ceramide synthesis *de novo* [32].

A study that surveyed the level of CB2 receptors in biopsies of human astrocytomas and glioblastomas revealed a high level of this receptor subtype among adult and pediatric tumors [33], and its amounts appeared to correlate to tumor malignancy. The high levels of CB2 expression suggest that these tumors would be vulnerable to cannabinoid treatment and indicate a potential CB2 agonist-based strategy. In contrast to these data, Held-Feindt *et al.* failed to demonstrate any relationship between CB1/CB2 receptors level expression and malignancy [34].

The suggestion that compounds specific to the CB2 receptor may be useful in glioma treatment. The hypothesis of the use of CB2 selective compounds has prompted further research on the effectiveness of a series of novel CB2 receptor compounds [35]. The lead compound named KM-233 represents the first generation of synthetic C1' aryl substituted cannabinoid ligands in human astroglial tumors. This compound exhibits good lipophilicity and a high affinity for the CB2 receptor that indicates significant transit across the BBB and good activity at the CB2 receptor on glioma cells. KM-233 showed excellent cytotoxicity against U87, U373 and C6 glioma cells and it was also effective *in vivo* in reducing glioma tumor growth in SCID mice via both direct intratumoral injection and systemic administration.

Although clinical trials are needed to definitely assess the efficacy of CB2 agonists, the *in vitro* and *in vivo* animal studies indicate that these ligands are promising tools with which to inhibit the growth of glial tumors.

Non-CB1/CB2 cannabinoids & gliomas

Another strategic approaches that has been pursued is to explore the use of natural nonpsychoactive cannabinoids that bind

cannabinoid receptors with very low affinity, thus excluding either psychotropic and/or immune/peripheral effects. Among the bioactive constituents of marijuana, cannabidiol (CBD) does not have significant intrinsic activity over cannabinoid receptors [3,36] and does not induce psychotropic or adverse side effects. For these reasons, it is one of the natural cannabinoids with the highest potential for therapeutic use. Massi *et al.* reported that CBD was effective in inhibiting U87 and U373 human glioma cells proliferation in an *in vitro* set of experiments [37]. Additional studies demonstrated the antitumor activity of CBD *in vivo* [37]. When tumor xenograft generated by subcutaneous injection of glioma cells in the flank region of immune-deficient mice were locally treated with CBD, there was a significant 60% mean reduction in tumor growth over a 23-day period of observation, although, in any case, complete eradication was observed [37]. The antiproliferative effect of CBD was dose correlated and dependent on its ability to induce apoptotic death. All these effects appeared to be independent of cannabinoid receptor stimulation [37].

Finally, also the synthetic derivative of THC, ajulemic acid, has been reported to inhibit glioma cell growth *in vitro* and *in vivo* thereby inducing cytostatic rather than cytotoxic effects [38], although its pharmacological properties are still controversial and not fully clarified.

Endogenous cannabinoids & gliomas

The enhancement of cannabinoid receptor expression in malignant versus healthy tissues described in a number of studies [32,33] has suggested a possible role of the endocannabinoid system in the tonic regulation of cancer cell growth. Gliomas have been shown to possess one or more components of the endocannabinoid system, such as the ability to synthesize endocannabinoids, the presence of CB1 and/or CB2 receptors, AEA membrane transporter, the enzyme fatty acid amidohydrolase (FAAH), which inactivates the endocannabinoids, thus suggesting a possible role of this system in the control of cell proliferation [4]. However, other than the finding of alterations of anandamide and/or 2-AG levels in some tumors, as well as the level of CB1/CB2 cannabinoid receptors compared with the corresponding healthy tissues, no unequivocal evidence has been reported so far to support this hypothesis [39–41].

Ongoing research is now evaluating whether endogenous cannabinoids exert tumor-suppressing effects in glioma growth, thus potentially representing an alternative approach for the development of possibly harmless anticancer drugs [4]. In fact, endogenous cannabinoid agonists or selective inhibitors of endocannabinoid degradation with limited action on CB1 receptors, would exhibit little if any psychotropic activity and would only be effective in tissues where the levels of endocannabinoid are altered. However, the antitumor potential of substances that modulate the endocannabinoid system is still largely unexplored.

The use of AEA would have a number of additional advantages over THC:

- AEA is virtually ineffective on CB2 receptors, which would rule out the immunosuppressive effect described for THC.
- AEA has been shown to promote the growth of hematopoietic cell lines, an effect which may be particularly attractive if AEA-enhancing strategies were to be included in polychemotherapeutic protocols

By contrast, the poor stability and short half-life of AEA makes its use as a therapeutic agent largely impractical. However, since a number of tumor cell lines express one or more component of the endocannabinoid system, and since AEA is synthesized on demand at multiple sites throughout the body and its lipophilic feature enables it to easily reach tumor sites, (including the CNS [42]), novel antiproliferative strategies based on the pharmacological modulation of AEA level through inhibition of AEA uptake and/or degradation by FAAH (an approach that would interfere with endocannabinoid levels mildly and in a neuronal activity-dependent fashion) may be considered for the clinical management of at least some forms of neoplastic disease.

Studies exploring on the putative antitumor properties of AEA in human glioma are only just beginning. It has been demonstrated that AEA induces apoptosis in cells derived from the neural crest, such as the CPH100 human neuroblastoma cell line, through a pathway involving increases in intracellular calcium, mitochondrial uncoupling and cytochrome c release [43]. Unlike AEA, other endocannabinoids such as 2-AG, linoleylethanolamide, oleylethanolamide and palmitoylethanolamide were unable to promote cell death [43]. Jacobsson *et al.* [44] showed that in rat C6 glioma cells, AEA exerts antiproliferative effects associated with a combined activation of cannabinoid and vanilloid receptors but, in contrast with Maccarrone's data [43], 2-AG inhibited glioma cell proliferation with a similar potency to that of AEA. Another endocannabinoid, stearoylethanolamide (SEA), which is present in the human brain in amounts comparable with those of AEA, induced a proapoptotic activity in glioma cell lines [45]. Contassot *et al.* demonstrated that human glioma cell lines, either established for a very long time (U87 and U251) or derived from a tumor biopsy (Ge227 and Ge258), are efficiently killed by AEA [46]. These cell lines express contemporarily CB1, CB2 and vanilloid receptor (VR)1 and the authors demonstrated that the antiproliferative effect of AEA was essentially due to its ability to bind the VR1 receptor.

Despite the scarce available data, the selective targeting of VR1 and/or CB1/CB2 receptors by endocannabinoid system modulation, could represent an attractive area of drug development, avoiding CB1-mediated psychotropic side effects and CB2-mediated immunosuppression.

Cellular mechanisms of the antitumoral action of cannabinoids

Progress has been made toward the understanding of the intracellular events underlying the *in vivo* and *in vitro* antitumor effects of cannabinoids. It is now established that in tumoral

cells, THC induces a considerable intracellular accumulation of *de novo*-synthesized ceramide that induces apoptosis. Of further interest is the demonstration that ceramide levels are inversely correlated with malignant progression of human glial tumors and poor prognosis [47].

A number of the downstream effectors of ceramide-mediated apoptosis have been recently characterized in cannabinoid-treated glioma cells *in vitro* and *in vivo* [25]. For example, *de novo*-synthesized ceramide provokes the rapid expression of p8, a transcription factor which in turn up-regulates two endoplasmic reticulum stress response-related transcription factors (ATF4 and CHOP). These proteins enhance the expression of the stress-regulated pseudokinase TRB3. The action of this and other proapoptotic proteins might converge in the mitochondria to trigger the intrinsic apoptotic pathway and the activation of executioner caspases [25]. In view of the emerging role of ceramide in the control of cell fate, the cannabinoid–ceramide connection might have exciting physiological implications and therapeutic possibilities.

However, it has been reported that mechanisms other than the ceramide–p8 pathway can be involved in cannabinoid-induced cell death. A recent study demonstrated that CB1 receptor may activate the JNK pathway through the $\beta\gamma$ subunits of the Gi protein, which may lead to the activation of Ras or Rac upstream of the JNK cascade [6]. JNK in many cases is involved in cell death and in C6.9 glioma cells, the remarkable increase in JNK activity caused by THC is accompanied by a significant decrease in cell viability.

In addition, it has been shown that the synthetic cannabinoid WIN-55, 212–2 inhibits proliferation and induces apoptosis in C6 glioma cells through the downregulation of ERK1/2 kinase and AKT, the key mediator of growth factor-promoted cell survival [48]. The downregulation of these intracellular pathways mediating the mitogenic/prosurvival signaling precedes reduction of Bad phosphorylation and the events that could follow Bad translocation to the mitochondrial membrane. Bad, a proapoptotic Bcl2 family member, may be an important link between the downregulation of the survival pathway and caspase activation evoked by cannabinoid treatment and resulting in glioma cell death [48].

The contribution of CB1 versus CB2 receptors in the antiproliferative effects on glioma cells remains unclear. In some cases, cannabinoids act through both receptors [22] and in others, through CB1 or CB2 alone [32,37,38,49]. Moreover, in some papers, the presence of a receptor-independent mechanism or even the involvement of a new unidentified cannabinoid receptor has been suggested [37,38,50].

The endogenous cannabinoids, AEA and 2-AG, share some of the cellular effects described for THC and synthetic cannabinoids, for example, inducing apoptosis and the acute increases in JNK and PKB activities; however, this proapoptotic effect, at least in some cases, appears to occur in spite, rather than because of classical cannabinoid receptor activation [51]. The involvement of other non-cannabinoid receptor-mediated events is confirmed by the observation that both JNK and PKB activation by AEA are only partially inhibited by the CB1 antagonist SR141716 [6,46].

Additional cellular mechanisms through which cannabinoids can modulate cell survival/death fate have recently been discovered. AEA was shown to inhibit tumor cell proliferation by inducing oxidative stress and intracellular calcium elevation, and its effect can be completely abrogated by α -tocopherol (an antioxidant) and calpeptin (a calpain inhibitor) [44]. CBD, a nonpsychotropic cannabinoid, is capable of killing glioma cells by causing apoptosis not associated with ceramide production and triggering an early production of reactive oxygen species and marked depletion of glutathione content [27,37]. Thus, one can conclude that oxidation pathway is very important and may be one of the main mechanisms of cannabinoid action in cancer cells.

Finally, substantial evidence has been collected supporting a fundamental role for lipoxygenase (LOX)- and COX-catalyzed arachidonic acid metabolism in cancer development. In particular, 5-LOX [52] and COX-2 [53] are the isoenzymes most involved in the control of cell growth and death within the CNS. The endocannabinoid SEA induces apoptosis in C6 glioma cells through activation of COX and LOX without involving MAPK cascades [45]. Moreover, *in vivo* treatment of nude mice bearing subcutaneous glioma tumor with CBD was found to significantly inhibit the activity and content of the 5-LOX enzyme in tumor tissues by 40% [MASSI ET AL., SUBMITTED]. These data provide further demonstration of other and/or alternative intracellular targets that can be modulated by cannabinoids, thus contributing to their evident antitumoral effect.

Cannabinoids & inhibition of tumor angiogenesis & migration

Cancer cell invasion and angiogenesis are crucial events in local spreading, growth and metastasis of tumors. New vascular formation represents a critical point that permits tumor development. Thus, angiogenesis is a promising target for the development of effective strategies for the treatment of malignant tumors in that it has the potential to starve large tumors and prevent the regrowth of residual margins. Among all types of cancer, gliomas are among the best vascularized human tumors. The fact that microvascular proliferation cannot be observed in low-grade gliomas has led to the assumption that both the development and progression of malignant gliomas largely depend on angiogenesis, representing a crucial pathophysiological step in this tumor entity. Complex and diverse cellular actions are implicated in angiogenesis, such as proliferation and migration of endothelial cells, extracellular matrix degradation and morphological differentiation of endothelial cells to form tubes. All these processes require a finely tuned balance between stimulatory and inhibitory signals (e.g., growth factors, VEGF, integrins, angiopoietins, chemokines, oxygen sensor and many others factors). Therefore, targeting neoangiogenesis may constitute one of the most promising therapeutic approaches against cancer/glioma.

With regard to this, there are some data showing that cannabinoids inhibit angiogenesis in different models. In a mouse model of glioma, Blazquez *et al.* observed that local administration of the JWH-133 caused altered blood vessel morphology in subcutaneous flank inoculated mice with either C6 glioma or human grade IV astrocytoma cells [54]. Later, the same group demonstrated that administration of different cannabinoids (THC, WIN-55,212-2, selective CB2 agonist JWH-133 and AEA) impairs the VEGF pathway either in glioma cells or in mouse bearing subcutaneous glioma [55]. The expression of various VEGF pathway-related genes was lowered. Cannabinoids decreased the production of VEGF and the activation of VEGF receptor (VEGFR)-2; both effects were abrogated either *in vitro* and *in vivo* by pharmacological blockade of ceramide biosynthesis. These changes in the VEGF pathway were paralleled by changes in tumor size [55]. Furthermore, intra-tumoral administration of THC to two patients affected by GBM was reported to decrease both VEGF level and VEGF receptor activation in tumors [55].

In addition, some studies have indicated that cannabinoids can play a role in cell migration. The cannabinoids compounds WIN 55,212-2 and JWH-133 inhibit the migration of the vascular endothelial cells HUVEC [54], thus indicating another feasible target of cannabinoids for antiangiogenic strategy. Moreover, CBD has been shown to inhibit U87 glioma cell migration in a dose-dependent manner [56]. The effect of CBD on cell migration appears to be very sensitive since lower doses are required to inhibit glioma cell migration than for inducing apoptosis [56]. More recently, Blazquez *et al.* demonstrated that local administration of THC in mice bearing subcutaneous gliomas downregulated the expression of tissue inhibitors of metalloproteinases (TIMPs) [57]. The cannabinoid-induced inhibition of TIMP-1 expression was mimicked by the selective CB2 compound JWH-133; it was abrogated by fumonisins B1, a selective inhibitor of ceramide synthesis *de novo* and, more interestingly, it was evident in two patients with recurrent GBM [57]. TIMP-1 inhibition by THC was also evident in cultures of various human glioma cell lines, as well as in primary tumor cells obtained from GBM patients [57]. Given the association between TIMP upregulation and tumor malignancy, the ability of cannabinoids to inhibit TIMP-1 could represent a further tool in comparing tumor invasiveness.

Finally, the recent demonstration that cannabinoids possess the ability to inhibit gliomagenesis appears to be very relevant. Neoplastic transformation of differentiated glial cells was the most accepted hypothesis to explain the origin of glioma for many years; however, recent findings support the existence of a stem cell-derived origin for different types of cancers such as gliomas, hematopoietic, breast and prostate tumors. In particular, glioma-derived stem-like cells (GSCs) have been isolated from both human brain tumors and several glioma cell lines [58–60]. GSCs constitute one of the potential origins of gliomas and, therefore, their elimination is an essential factor for the development of efficient strategies. Guzman's group provided

evidence that the synthetic cannabinoid HU-210 (a non-selective cannabinoid agonist) and/or JWH-133 (a CB2 agonist) significantly altered the expression of genes involved in the regulation of stem cell proliferation and differentiation, and decreased the efficiency of GSCs to initiate glioma formation *in vivo* [61].

Selectivity of cannabinoid action on tumor cells

Antitumor compounds should selectively affect tumor cells. It appears that cannabinoids are able to do this, as they kill tumor cells but do not affect their nontransformed counterparts.

In contrast to the conventional cancer chemotherapies, cannabinoids do not exhibit the typical pan-toxicity associated with most chemotherapeutic agents, and have the ability to easily cross the BBB. The apparent selective efficacy shown by cannabinoids is of potential clinical relevance. The THC-induced cell death observed in rat C6 glioma cells did not occur in nontransformed neurons or in rodent primary astrocytes [21]. *In vivo* cannabinoids inhibit, and in part eradicate, C6 glioma tumors with no discernable tissue damage in surrounding healthy tissue [22].

The difference in sensitivity could be ascribed to differences in signal transduction events downstream, the cell surface and/or compared with different redox state cellular feature of the tumoral cells versus normal cell. The first hypothesis is supported by papers by Carracedo's where it has been shown that cannabinoid treatment does not trigger neither ceramide accumulation, upregulation of p8 nor other ER stress-related genes in nontransformed astrocytes [24,25]. The oxidative hypothesis is consistent with the finding that oxidative metabolism and associated sensitivity to apoptosis in tumor cells differ from normal cells [62,63].

This selective property is shared by CBD, which reduced glioma cancer cell growth with no apparent toxicity on non transformed cell counterparts, as demonstrated by Massi *et al.* [27]. More recently, Dunsch *et al.* [35] documented cells the selective efficacy of the CB2 cannabinoid agonist KM-233 in U87 glioma. In addition, other groups have demonstrated its efficacy in non brain-derived cells [64,65].

To summarize, it can be proposed that this dual property may depend on differences in the features of normal versus transformed cells, and that an opposite regulation of CB1 and CB2 expression on tumoral versus normal cells could justify the different sensitivity to cannabinoids. This does not seem to be an univocal explanation because, besides the demonstration of a receptor-independent effect induced by cannabinoids in glioma cell death [21,27,37], neither glioma cells or glial cells possess both cannabinoid receptors [66,67]. This cannabinoid selectivity, if definitively proved for additional tumor cell lines, might be of considerable therapeutic interest.

Cannabinoids & increased risk of cancer

Despite the suggested antitumoral effects of cannabinoids, it has also been suggested that cannabinoids could promote the development of cancer. Epidemiological studies investigating

the relationship of cannabis smoking and various forms of cancer have yielded inconsistent results, thus failing to resolve the conflicting findings in animal models of cancer or in cancer cell lines. An inconsistent association between cannabis smoke and cancer has been reported, and administration of high oral doses of THC in rats or mice did not increase tumor incidence in a 2-year study [68]. In animal models, cannabinoids exert a direct antiproliferative effect on tumors, but they could indirectly enhance tumor growth via immune system inhibition, imposing caution for any possible further studies. The immune suppression induced by cannabinoids [69] could enhance cell proliferation and accelerate cancer progression in patients [70,71]. Hart *et al.* have demonstrated that treatment of lung cancer squamous cell carcinoma, bladder carcinoma, glioblastoma (U373-MG), astrocytoma and kidney cancer cells with nanomolar concentrations of cannabinoids such as THC, anandamide, HU-210 and WIN 55,212-2 leads to rapid epidermal growth factor receptor- and metalloprotease-dependent cancer cell proliferation [72]. However, the same study also documented that at micromolar concentrations, cannabinoids induced cancer cell apoptosis, in agreement with previous reports. These results highlight the bimodal action of cannabinoids on cancer cell growth, with low concentrations being proproliferative and high concentrations having antiproliferative effects.

The variability of the effects of cannabinoids in different tumor models may be related to the differential expression of CB1 and CB2 receptors. Thus, cannabinoids may be effective in killing tumors that abundantly express cannabinoid receptors, such as gliomas, but may increase the growth and metastasis of other types of tumors, such as breast cancer, with no or low expression of cannabinoid receptors, owing to the suppression of the antitumor immune system [71].

Clinical application of cannabinoids in gliomas

With many anticancer agents, the encouraging results obtained with cannabinoids against gliomas in *in vitro* or *in vivo* studies do not guarantee their efficacy in humans.

Given the favorable safety profile, in March 2002, the Spanish Ministry of Health approved a Phase I/II clinical trial, which was carried out in collaboration with the Tenerife University Hospital and Guzman's research group, aimed at investigating the effect of local administration of THC on the growth of recurrent GBM. The study was the first pilot study investigating the antitumoral actions of cannabinoids but also the intracranial application of THC through an infusion cannula connected to a subcutaneous reservoir. The nine enrolled patients had previously failed standard therapy (surgery and radiotherapy) and constitute a cohort of terminal patients harboring actively growing recurrent tumors. The results have been recently published [73] and THC delivery was shown to be safe and with no overt psychoactive effects. Median survival of the cohort from the beginning of cannabinoid administration was 24 weeks, and two patients survived for approximately 1 year.

Survival for GBM patients following diagnosis is typically 6–12 months and the authors reported that owing to the characteristic of the study, the effect of THC on patient survival was unclear, and evaluation of survival would require a larger trial with a different design. In placebo-controlled trials for recurrent GBM treated with temozolomide, a slight impact on overall length of survival (median survival: 24 weeks; 6-month survival rate: 46–60%) was reported [74,75]. Given the possibility that cannabinoids can increase cancer risk, the authors underlined that the study clearly demonstrated that THC did not facilitate tumor growth or decrease patient survival. Although the trial results were encouraging, the authors admitted that THC was not the most appropriate cannabinoid agonist for antitumoral strategy owing to its high hydrophobicity and relatively weak agonistic potency. Unfortunately, at present other attractive compounds, such as JWH-133 or WIN-55,212-5, cannot be used in trials owing to a lack of thorough preclinical toxicology studies.

Expert commentary

The cannabinoid system has the potential to provide a therapeutic target for tumor intervention in cancers such as malignant gliomas and astrocytomas, as well as other types of cancers and as breast, thyroid, pancreatic, prostate and skin cancers. In a series of experimental research studies, cannabinoids have been shown to exert their antitumoral action by inducing apoptosis or blocking proliferation, angiogenesis and tumor invasion. This evidence, together with the very low toxicity of cannabinoids compared with other chemotherapeutic agents, make these compounds promising tools for the management of gliomas. However, in general, one must be cautious when envisaging the potential clinical use of new anticancer therapies. Despite the large amount of literature on how the pathophysiology of cancer cells, there has been no parallel advance in the clinical practice of chemotherapy. Many compounds that inhibit cancer cell growth in culture and in animals models display ineffective properties and/or toxic effects when tested in patients.

With regard to cannabinoids, at present, they have shown notable antitumor activity in different animal models of glioma, but their possible antitumor effects in patients have not been well established. The only human clinical trial on gliomas that has been conducted attempted to confirm whether THC has potential as a therapeutic agent [73]. However, the results were inconclusive and did not outline in an unequivocal manner the real advantage of cannabinoid use. Moreover, in that trial [73], another critical point was the route of administration (intracranial application of THC through an infusion cannula), which cannot be considered an easy and suitable method to use, although it enabled THC to reach high levels within the tumor and to reduce adverse side effects. The latter consideration raises the more general question about the best route of cannabinoid administration for therapeutic application. In fact, cannabinoids are poorly soluble in water, determining their pharmacokinetics

behavior in terms of absorption/distribution, and it could be one of the difficulties in formulating the preparations of pure compounds for medicinal use and for finding appropriate route of delivery. Synthetic water soluble cannabinoids (such as the compound O-1057) might help overcome some of these pharmacokinetic peculiarities and enable the development of suitable preparations for oral or intravenous administration.

Thus, in these scenarios, the effectiveness of cannabinoids against cancer cell growth have to be conclusively proven *in vivo* and/or in adequate clinical trials. Since medicine has been unsuccessful in controlling gliomas growth for several decades, we believe that it will be necessary to use selective strategies, such as the combination of several drugs and/or cannabinoids, as well as other therapeutic approaches to achieve a significant improvement in the treatment of this devastating disease.

Five-year view

Even if the first partial result obtained from glioma clinical trial has no shed light on the real effectiveness of THC treatment in patients, the fair safety profile of cannabinoids together with their demonstrated antiproliferative action in animals and in *in vitro* studies on tumoral cells may set the basis for future research aimed at better exploring the antitumoral activity of these compounds. Additional experimental studies and trials are necessary to determine whether cannabinoids as single drugs or in combination with established antiglioma drugs, could be used other than for their palliative effect, to inhibit tumor growth. Suggestions for these potential new studies can be found below.

Molecular & cellular studies

As with many other antitumor agents, further research on cannabinoids is required to evaluate the precise mechanism of anticancer action of cannabinoids. If we can better understand the intracellular signaling pathways, the processes in which they are involved and their sensibility or resistance to cannabinoids, it will be one step closer to understanding whether these compounds could be used in clinical paradigm and how.

Natural, synthetic, endocannabinoid compounds & hybrid drugs

Since THC is probably not the most likely appropriate cannabinoid agonist for future antitumoral strategies owing to its high hydrophobicity, relatively weak agonistic potency and ability to elicit CB1-mediated psychotropic effects, it would be desirable to test other less hydrophobic CB1/CB2 mixed agonists. Moreover, since CB2 cannabinoid agonists have been shown to possess strong efficacy in *in vivo* and *in vitro* tests, it could be of interest to test their real efficacy. Furthermore, nonpsychotropic cannabinoid compounds such as cannabidiol or the synthetic ajulemic acid have been shown to possess anticancer properties. Thus, it will be necessary to test their capacity to reduce tumor growth.

A more comprehensive understanding of the role of the endocannabinoid system in the regulation of tumor growth is also required. Future research should address the question of whether or not endogenous cannabinoids exert a real tumor-suppressing effect with the aim of developing harmless anticancer drugs. In fact, selective modulators of the endocannabinoid system with no direct action on CB1 receptors would exhibit little, if any, psychotropic effects and would be most effective in tissues where the levels of endocannabinoids are pathologically altered.

Cannabinoids in combination with other chemotherapeutic drugs

To date, the success of chemotherapeutic treatments of gliomas have been hampered by factors such as rapid growth, high degree of infiltration and extreme resistance to chemotherapy. It is therefore conceivable that a combined therapy of a cannabinoids with a classical therapy already in use, could provide better results. Recent experimental data from a study by Carracedo *et al.* showed the synergic effect of cannabinoids with different antitumoral drugs (e.g., cisplatin and doxorubicin), as well as with endoplasmic reticulum-stress inducers (tunicamycin and thapsigargin), strongly suggesting that cannabinoids could be used in combination with well-established therapies [25]. For example, cannabinoids plus temozolomide and/or other agents might exert a more potent clinical impact than cannabinoids or temozolomide alone. Finally, the effectiveness of a possible synergism between cannabinoids and other clinical treatment such as radiotherapy needs to be explored.

Moreover, given the current importance of COX and LOX enzymes in the development/progression of tumors in many forms of cancer, it will be of interest to test the efficacy of cannabinoids in combination with COX and LOX inhibitors.

Cannabinoids in combination with antiangiogenic treatment

Since antiangiogenic molecules currently represent the most promising and complementary approach in anticancer therapy, a clinical trial that assessed the effectiveness of cannabinoids to reduce tumor growth by limiting angiogenic processes would be of relevance. Antiangiogenic therapy is considered to be an alternative or complementary paradigm to conventional cancer treatments. In view of positive experimental results recently obtained with cannabinoid it would be planned to study either *in vitro* or *in vivo*, studies exploring whether cannabinoids could be used alone or in combination with molecules that inhibit the pro-angiogenic features of tumors and thus improve the glioma clinical outcome would be of great interest.

Standardization of cannabinoid administration

As already reported for other chemotherapeutic agents, it is conceivable that a better cannabinoid administration regimen would ameliorate the clinical results. Thus, new clinical trials with a cannabinoid-based medicine in newly-diagnosed glioma patients would be desirable to obtain better outcomes.

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Key issues

- Gliomas are the most frequent class of malignant primary brain tumors and one of the most aggressive forms of cancer, with high invasiveness, high-proliferation rate and rich neovascularization. Present therapeutic strategies for their treatment (surgery, radiotherapy and limited chemotherapeutic drugs) are usually inefficient and in most cases, just palliative.
- Cannabinoids, the active compounds of *Cannabis sativa*, and their synthetic derivatives act in the humans by mimicking endogenous substances termed 'endocannabinoids' that activate specific cannabinoid receptors (CB1 and CB2).
- The best-established therapeutic effect of cannabinoids in cancer patients at present is the attenuation of chemotherapy-induced nausea and vomiting, with evidence of their possible use in appetite stimulation and pain inhibition.
- Cannabinoids inhibit glioma tumor growth either *in vitro* or *in vivo* models through several cellular mechanism such as elevating ceramide levels, inducing stress state in tumoral cells, arresting cell cycle and inducing apoptosis.
- Cannabinoids inhibit angiogenesis and reduce the invasiveness of glioma.
- Cannabinoids kill tumor cells without toxicity on their nontransformed counterparts, probably modulating the cell survival/cell death pathways differently.
- Cannabinoids show a favorable drug-safety profile and do not exhibit important toxicity as the conventional chemotherapeutic drugs.
- Recently, in 20% of terminal patients with recurrent glioblastoma multiforme, tetrahydrocannabinol prolonged the survival time; in the remaining patients, the cannabinoid did not facilitate tumor growth or decrease survival.
- Additional basic and clinical research are needed to clarify the mechanism of cannabinoid action, to find new natural, synthetic cannabinoids and/or hybrid molecules active against gliomas for use alone or in combination with other chemotherapeutic agents.

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